Formulation, Characterisation and Antiproliferative Effects of Boswellic Acids Loaded Chitosan Nanoparticles on Human Lung Cancer Cell Line A549

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INTRODUCTION

Cancer worldwide continues to grow despite advances for diagnosis and its treatment. Among all types of cancer, lung cancer still ranks on the top as the leading cause of deaths due to cancer in humans, with more than 1.7 million deaths each year worldwide [1]. Boswellic acids (BAs) are pentacyclic terpenoids isolated from oleo gum resin of *Boswellia serrata* has shown promising antiproliferative and anticancer effects but allied with poor water solubility and bioavailability constraints [2]. Nanoparticles in the field of nanomedicine have been evolving as vital carriers by offering different drug targeting approaches for the effective delivery of conventional chemotherapeutic agents in cancer management [3]. This nanocarrier approach protects active drug molecules from devastating effects of physiological fluids ultimately leading to minimization of drug dosage, sustainable drug release and enhanced cellular uptake with significant accumulation inside the cancerous tissues. Currently, anticancer investigations have been focused on biodegradable polymeric NPs because sustained release cross-linked polymeric NPs enable enhancement of aqueous solubility due to nano-sized particles with significant bioavailability improvements by protecting drugs from the gastrointestinal tract [4-5].

AIM

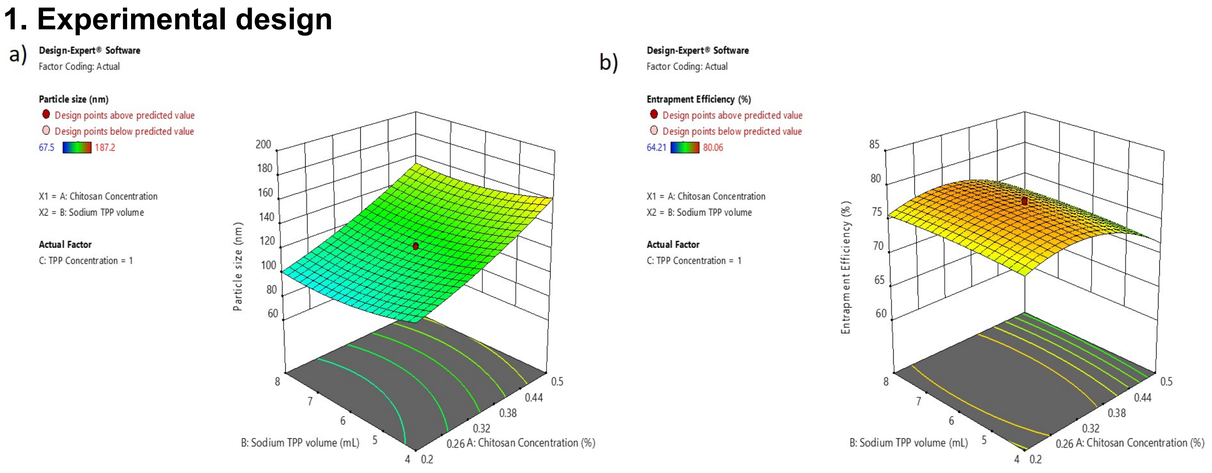
The main aim of the research was to formulate BAs loaded chitosan nanoparticles followed by characterisation and evaluation of *in vitro* release. The developed formulation was evaluated on human lung cancer cell line A549.

METHODS

BAs loaded chitosan nanoparticles were synthesised using ionic gelation technique. The influence of independent variables were studied and optimised on dependent variables using central composite design. The cytotoxic effects of free BAs and BAs loaded CNPs on Human Lung cancer (A-549) cell lines was evaluated by MTT assay. Optimum formulation obtained after statistical optimization was further explored to find out cell death confirmation by apoptotic studies especially DNA ladder assay and cell cycle analysis by Flow cytometry.

RESULTS

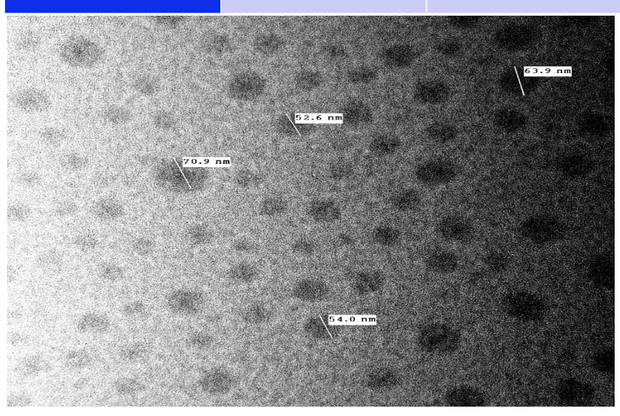
1. Experimental design



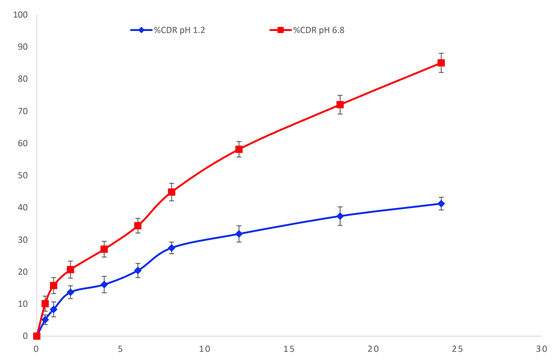
3D response surface plot indicating the effect of different variables on (a) particle size and (b) entrapment efficiency

2. Physico-chemical characterization

	Z- average	Polydispersity index (PDI)	Loading capacity	Entrapment efficiency (EE) %
BAS CNPs	67.5±0.2 nm	0.147± 0.02 nm	77.6±0.6 nm	80.1±0.4

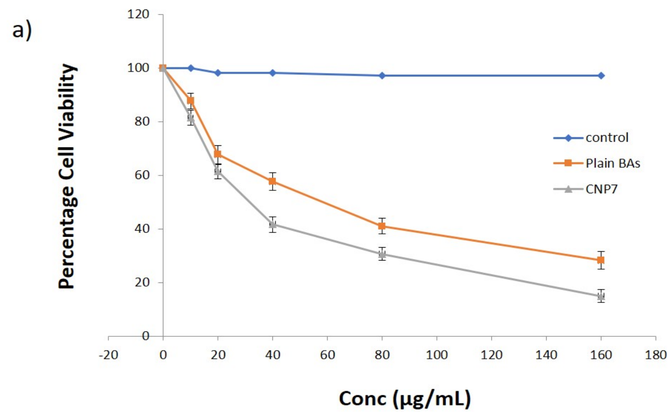


TEM Image of BAs loaded chitosan nanoparticles (100nm)



In-vitro release of BAs loaded chitosan nanoparticles in pH 1.2 and 6.8

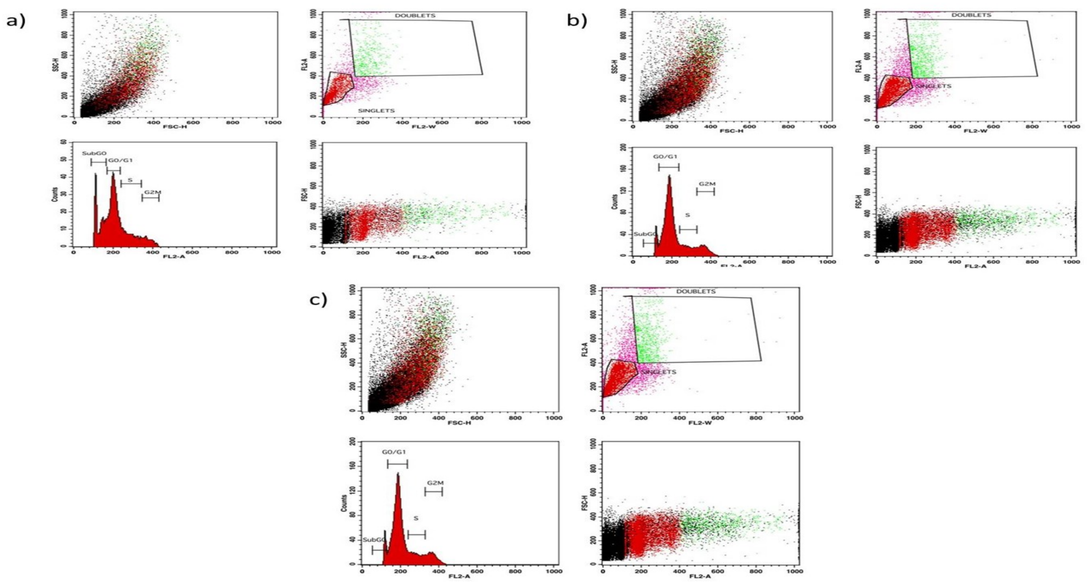
3. Cytotoxic Studies



a) MTT assay

b) Trypan **blue staining**

Cell cycle analysis



a) Control A-459 cells b) A-459 cells treated with 20 µg/mL of sample c) A-459 cells treated with 40 µg/mL of sample

DISCUSSION

In the current study, we made an effort to fabricate the BAs loaded chitosan nanoparticles using ionic gelation technique and explore their efficacy *in vitro* in lung cancer cell line A549. The influence of independent variables, chitosan concentration (X1), Sodium tripolyphosphate (NaTPP) volume (X2) and tripolyphosphate concentration (X3) were studied and optimised on dependent variables (particle size and entrapment efficiency) using central composite design. The optimised formulation were observed spherical in shape with excellent entrapment efficiency (80.06 ± 0.48), showed sustained release at pH 1.2 and maximum release at pH 6.8. Drug encapsulation can be attributed to physical entrapment or chemical conjugation. Polydispersity index (PDI) represents the size distribution of and uniformity of nanoparticles. The positive reading of zeta potential indicates that there is stable dispersion between particles which reduces the chances of aggregation. The minimum particle size of 67.5 nm having PDI value 0.147 (confirming monodispersity) was observed in formulation CNP7 at a lower value of chitosan concentration, a high value of NaTPP volume and high value of NaTPP concentration. The cytotoxicity assay revealed enhanced cytotoxicity for drug-loaded nanoparticles in contrast to the free drug having an IC50 value of 17.29 µM and 29.59 µM, respectively. Flow cytometry confirmed that treatment of cells with 40 µg/ml had arrested 22.75 ± 0.3 % at SubG0 phase of the cell cycle when compared to untreated A459 cells. The observed results justified the BAs loaded chitosan nanoparticles were effective due to greater cellular uptake, sustained intercellular drug retention and enhanced anti-proliferative effect by inducing apoptosis.

CONCLUSIONS

The study reveals that the BAs loaded chitosan nanoparticles prepared by using ionic gelation technique stimulate apoptosis in A549 cell line, exhibiting its potent cytotoxic activity. Nanoencapsulation process may also be assisting the entry of BAs into the cancerous cells, providing targeted effects and better patient compliance. Overall, our study highlighted the relevance of NPs of BAs in targeting lung cancer as an alternative treatment over the existing chemotherapy, providing a new direction to the cancer clinic. Furthermore, the findings can also be extended to various other phytochemicals and types of cancer.

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Acknowledgments

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